The Green Oat Story: Possible Mechanisms of Green Color Formation in Oat Products during Cooking

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ABSTRACT: Consumers occasionally report greenish colors generated in their oat products when cooking in tap water. Here we have investigated pH and ferrous (Fe^{2+}) ion as possible mechanisms for this color change. Steel-cut oat groats can turn brown-green color when cooked in alkaline conditions (pHs 9 to 12). Extraction of this color with methanol, and high-pressure liquid chromatography indicated a direct association of this color with the phenolic acid or avenanthramide content of the oat. The presence of 50 mM $NaHCO_3$ in water will cause oat/water mixtures to turn alkaline when cooked as CO_2 is driven off, generating OH^- ion. Although tap water rarely, if ever, contains so much bicarbonate, bicarbonate is used as a leavening agent in baking applications. Industrial interests using baking soda or alkaline conditions during oat processing should be aware of possible off color generation. We have also found that as little as 10 ppm Fe^{2+} will turn oat products gray-green when cooked. The aleurone stained darker than the starchy endosperm. Other divalent cations, such as Ca^{2+} or Mg^{2+} had no effect on cooked oat color. As much as 50 ppm Fe^{2+} may be found in freshly pumped well water, but Fe^{2+} reacts quickly with oxygen and precipitates as $Fe(OH)_3$. Thus, some freshly pumped well water may turn oats green when cooked, but if the water is left under atmospheric conditions for several hours, no discoloration will appear in the cooked oats.

Keywords: *Avena*, avenanthramides, bicarbonate, ferrous ion, iron

Introduction

Oat processors receive complaints at irregular intervals about their steel-cut groat products turning green when cooked in tap water. This type of report is particularly surprising, considering that oat groats contain no known pigments. The effect has been notoriously difficult to replicate in a laboratory, and thus no report of this phenomenon has ever appeared in the scientific literature (to our knowledge). The green color, which appears during cooking, provides a distinctly unpalatable appearance, and is thus undesirable. We have sought a mechanism for the appearance of the green color.

One hypothesis tested in our investigation was derived from knowledge of greenish color derived from sunflower meal at alkaline pH. Attempts to extract protein from sunflower meal at alkaline pH have resulted in green to brown colors, which have been attributed to phenolic compounds (Carter and others 1972), specifically chlorogenic, quinic, and caffeic acids that turn colors, ranging from green-brown to yellow under alkaline conditions (pHs 8 to 11). The discoloration of sunflower meal by these compounds at alkaline pH is considered a significant problem (Dorrell 1976).

Oats also contain significant concentrations of phenolic compounds, referred to as the avenanthramides (Collins 1989). Avenanthramides have been studied as to their presence and stability in oat-based food products (Dimburg and others 2001), and are of significant nutritional interest because of their antioxidant activity (Dimberg and others 1993; Emmons and Peterson 2001; Martenez-Tome and others 2004). The avenanthramides also turn greenish brown in alkaline conditions (Collins 1989). We considered it a possibility that the color observed by consumers could be derived from these phenolic compounds. Unfortunately, all reports of water sources turning oats green have been anecdotal, and we have not yet duplicated these effects from water sent to our laboratory from the field. In this study, we considered the possibility that cooking oats in hard water with high concentrations of bicarbonate ion might affect the pH of the cooked product, thus affecting the color.

Another possible mechanism by which tap water might cause off colors in cooked oat products involves iron. There is a significant interest in off colors caused by iron because of efforts to fortify food nutritionally with iron (Mellican and others 2003). Iron is also known to interact with the bran of wheat and corn (Reinhold and others 1981; Fernandez and Phillips 1982; Leigh and Miller 1983). Ferrous sulfate has been reported to turn the color of barley porridge to an unappealing gray (Theuer 2002). Iron can occur in concentrations of up to 50 ppm its reduced ferrous form (Fe^{+2}) in well water, but is most commonly found in concentrations of 10 ppm or less (Driscol 1986). When exposed to air, Fe^{+2} is rapidly oxidized to its ferric (Fe^{+3}) form by atmospheric oxygen and will precipitate out of solution as $Fe(OH)_3$, or some other salt (Benefield and Morgan 1990) according to the formula:

$$4Fe^{+2} + O_2 + 10H_2O \leftrightarrow 4Fe(OH)_3(s) + 8H^+$$

$Fe(OH)_3$ is soluble at levels 10^{-8} to 10^{-9} mol/L at neutral pH, thus precipitates readily. We have also considered in this study that Fe^{+2} might cause off colors in cooked oat products. We do

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not exclude the possibility that mechanisms other than those presented here may also turn oats green in food systems.

Materials and Methods

Steel-cut groat cooking experiments

Three experiments were conducted that involved cooking of steel-cut oat groats to study color generation that were of similar design but of differing objectives. Steel-cut oat (*Avena sativa* L.) groats were obtained from Grain Millers Inc. (St. Ansgar, Iowa, U.S.A.). In all experiments, 1 g of cut groats was placed in 15 mL screw-cap tubes, and mixed with 5 mL of a test solution. The oat/test solution mixtures were placed in a boiling water bath and cooked for 10 min. Other treatments differed in each of the 3 experiments. Chemicals were obtained from Sigma-Aldrich (St. Louis, Mo., U.S.A.).

The 1st cooked groat experiment tested the effects of pH on extractable color. The test solution was a buffer consisting of 100 mM sodium pyrophosphate (Sigma-Aldrich nr 221368) and 100 mM sodium tetraborate (Sigma-Aldrich nr B-0127) adjusted to pH 6, 7, 8, 9, 10, 11, or 12. After cooking, 10 mL of methanol were added to each tube to precipitate the starch and to extract the color. Tubes were then centrifuged at 500 × *g* for 10 min to settle particulate matter. The supernatants were then transferred to 50 mL polypropylene centrifuge tubes and centrifuged at 30000 × *g* for 50 min to clarify the extracts. Spectra of extracts were determined from 250 to 500 nm with a Cary 50 Bio UV visible spectrophotometer (Varian, Lake Forest, Calif., U.S.A.). Color photography of extracts was made with a digital camera under natural light. The camera was a Canon (Japan) model EOS D60 with a 100 mm macro lens.

The 2nd cut groat cooking experiment was designed to test the effect of bicarbonate concentration in the cooking solution upon the pH and color of the solution after cooking. For these experiments, the test solutions were 0, 1, 5, 10, 50, 100, 200, 500, and 1000 mM sodium bicarbonate (Sigma-Aldrich nr S-6014). The addition of methanol and the centrifugation protocols were identical to the previous experiment, but the pH of the clarified extract was measured with a pH meter, and the absorbance at 400 nm determined with the same spectrophotometer described previously.

The 3rd experiment tested the effect of ferrous ion on color in cooked groats. In this experiment the test solution was either water or 50 ppm Fe^{+2} (1.64 mM iron II sulfate hepahydrate, Sigma-Aldridge nr 215422, prepared in the absence of O_2). After cooking, groats were rinsed with distilled water and the color difference and pattern were recorded with digital photography under natural light. The same digital camera and lens described above were used for this image but with an additional 125 mm extension tube to allow the necessary magnification.

Oat paste cooking experiments

Two experiments were performed where we cooked oat flour with test solutions to determine the effect of test solutions on the color of uniformly blended oat flour. For these, we placed 4 g of oat flour in round metal pans 6 cm dia, with 6.75 mL of test solutions. The oat flour/test solution slurries were mixed well and spread to make a smooth surface. The lid of the metal container was closed and the mixtures were placed in a vegetable steamer over boiling water and the slurries were cooked at about 106 °C for 10 min. After cooking, the resulting gelled disk was removed and its color analyzed with a Minolta color meter.

In the 1st experiment, we tested the effects of pH on flour color. The test solution was a buffer consisting of 100 mM sodium pyrophosphate (Sigma-Aldrich nr 221368) and 100 mM sodium tetraborate (Sigma-Aldrich nr B-0127) adjusted to pH 6, 9, and 12. In the 2nd experiment, we tested the effects of divalent ion on color in the cooked flour. For this experiment, the test solutions were salt solutions prepared using $CaCl_2.2H_2O$, $Fe_2SO_4.7H_2O$, and $MgCl_2.6H_2O$ (Sigma Chemical Co., St. Louis, Mo., U.S.A.) at concentrations of 0, 10, 50, 100, 200, 300, 400, and 1000 ppm (based on cation's atomic weight). Ferrous sulfate solutions were prepared in the absence of oxygen using degassed water bubbled with nitrogen gas.

Minolta colorimeter measurements

Oat paste color was measured using a Minolta Chroma Meter CR-310 (Konica Minolta Sensing Americas Inc., Ramsey, N.J., U.S.A.) by directly placing the measuring head on the cooled paste gel. Color readings were expressed by Comission Internationale de l'Eclairage (CIE–Intl. Commission on Illumination) values for L^*, a^*, and b^*. L^*-values measured black to white (0 to 100); a^*-values measured redness when positive and greenness when negative; b^* values measured yellowness when positive. Also, these data from the Minolta were used to calculate hue angle and chroma values according to the method of Little (1975).

Avenanthramide analysis

Authentic avenanthramides (A, B, and C) were synthesized as described by Collins (1989). Total avenanthramide was extracted in 80% ethanol and analyzed by HPLC as described by Wise and others (2008). Briefly, extracts were dried by rotary evaporation and resuspended in 2 mL MeOH and filtered through a 0.22 μm filter. Ten-microliter aliquots were analyzed by high-pressure liquid chromatography (HPLC) on a 4.6 × 50 mm C-18 column using a fixed wavelength spectrophotometer detector at 340 nm. The mobile phase consisted of buffer A: H_20 with 5% acetonitrile and 0.1% formic acid and buffer B: acetonitrile with 0.1% formic acid. A gradient of 13% to 30% B over 20 min at a flow rate of 1 mL/min was employed.

To analyze the spectral properties of the eluant at different pH values, 1 mL fractions were collected (1 fraction/min). One-half milliliter of each fraction was mixed with 100 mM acetate buffer, pH 4 and the absorbance at 330 and 400 nm measured with a spectrophotometer. One-half milliliter of each fraction was also mixed with borate buffer, pH 10, and the absorbance at 400 nm measured.

Statistical analysis

All experiments were performed in triplicate. Analyses of variance were performed with the Statistix computer package (Analytical Software, Tallahassee, Fla., U.S.A.). Mean separation was carried with the least significant difference (LSD), also calculated with the Statistix computer package.

Results and Discussion

pH effect on color

Oats cooked at pH 6 had a typical beige color characteristic to oat cereal. Oat pastes cooked at pH 9 and 12 developed a more brown-green color (images not shown). Minolta color meter analysis of the paste gels indicated that the increase in pH was associated with a decrease in lightness, an increase in redness, and no change in yellowness. Chroma values did not change significantly with pH. Hue angle was highest at pH 6 and was significantly lower at pH 9 and 12 (Table 1).

The color that developed in steel-cut groats cooked at different pH values could be extracted with methanol. Extracts made at pH

6 were relatively colorless, whereas extracts cooked at pH 9 and 12 visually became more yellow-green in color (Figure 1). Spectra of these methanol extracts demonstrated distinctive shifts toward the visible region with increasing pH (Figure 2). Based on these spectra, we chose 400 nm at pH 10 as a characteristic wavelength and pH for a simplified diagnostic wavelength to further characterize this phenomenon.

We hypothesized that the observed yellow-green color observed in oat extracts at alkaline pH were due to phenolic compounds, such as the avenanthramides (Collins 1989). Spectra of synthetic avenanthramides A, B, and C were determined in the same buffers used in the cooking experiment to document the effect of pH on spectra of these individual compounds. These spectra (Figure 3) also showed shifts toward the visible range at alkaline pH, as suggested by Collins (1989). Although these spectra only show absorbance between 250 and 500 mm, scans were carried out to 700 nm, but data over 500 nm were deleted because there was no absorbance (or very little) at those wavelengths.

Total avenanthramide content was evaluated from 80% ethanol extracts by HPLC on a C-18 column (Figure 4). Three major peaks corresponded to synthetic standards for avenanthramide A, B, and C. Numerous other UV absorbing compounds were observed in the ethanol extract (Figure 4). Although their identity is not known, we suspect that these represent some of the 30 or more minor avenanthramide forms described by Collins (1989).

Table 1—Effect of pH on cooked oat paste color as measured by a Minolta color meter.

pH	*L**	*a**	*b**	Hue angle	Chroma
6.0	+63.8*a*	+1.26*b*	+25.3*a*	+87.2*a*	+25.3*a*
9.0	+59.5*b*	+2.95*a*	+26.8*a*	+83.7*b*	+26.9*a*
12.0	+53.3*c*	+2.60*a*	+25.9*a*	+84.3*b*	+26*a*
LSD	1.5	0.35	NS	0.9	NS

Values in the same column with the same letter do not significantly differ from each other ($P < 0.05$).

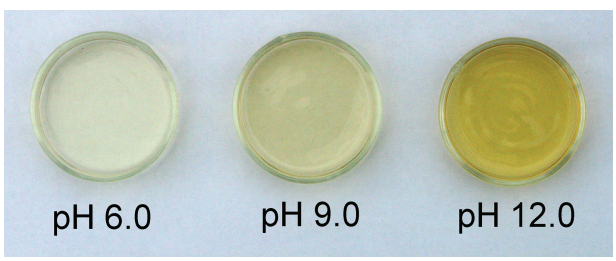

Figure 1—Effect of pH on color in methanol extracts of cooked oats.

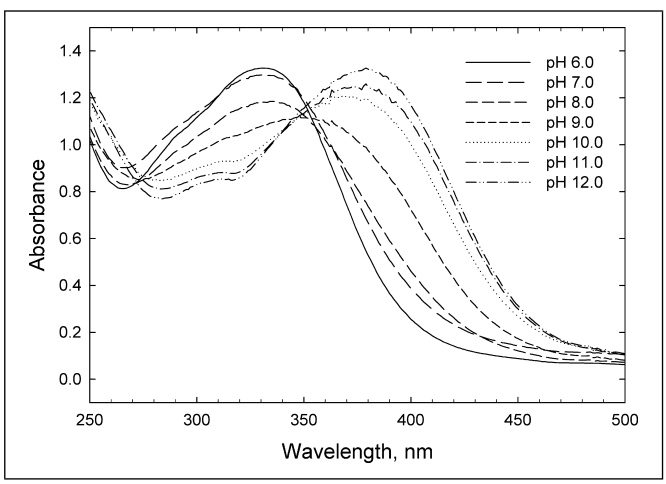

Figure 2—Spectra of methanolic extracts of oats cooked at pHs 6 to 12.

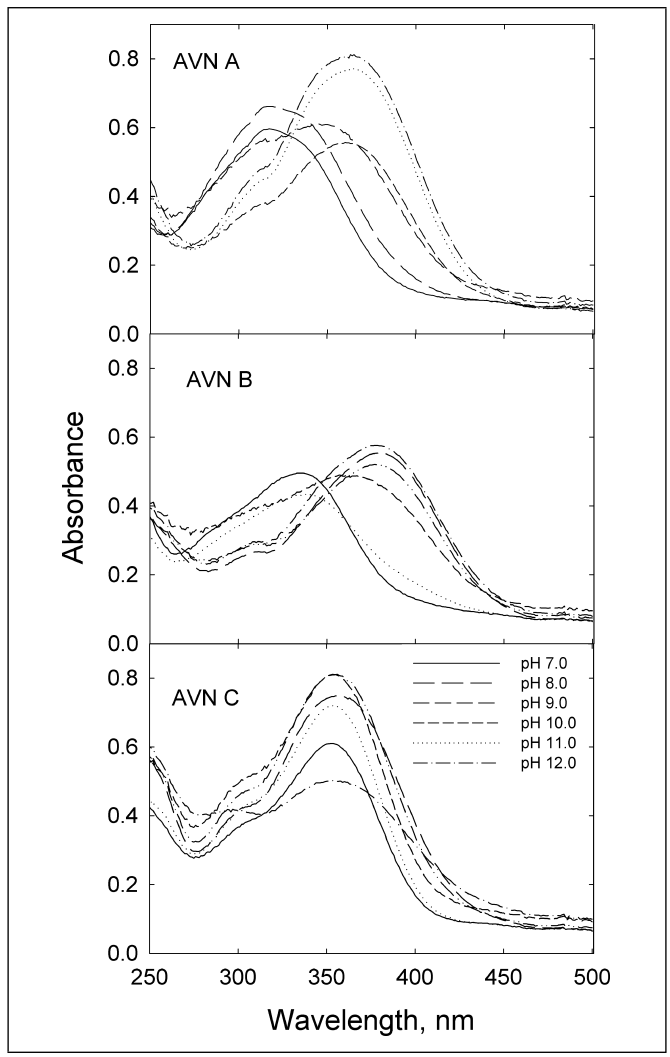

Figure 3—Spectra of avenanthramides (AVN) A, B, C at pHs 7 to 12.

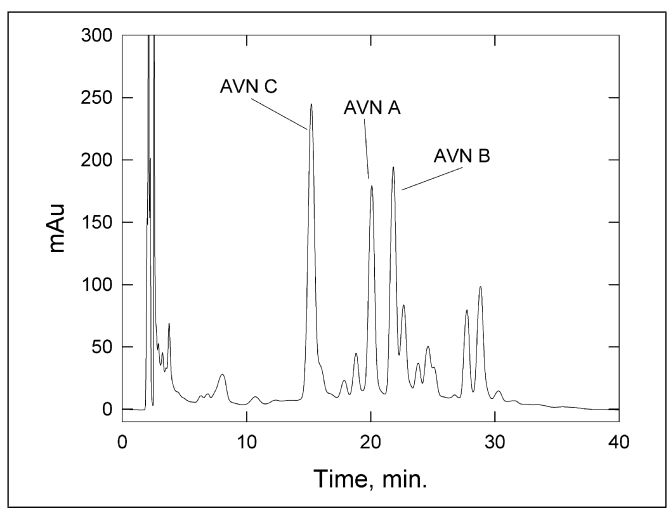

Figure 4—UV (340 nm) trace from 80% ethanol extract from C-18 HPLC.

To confirm that yellow-green color extracted from oats cooked in alkaline conditions was associated with the avenanthramides, we collected fractions from the HPLC and measured their absorbance (330 and 400 nm) at pH 4, and then their absorbance at 400 nm at pH 10. The UV absorbance of collected HPLC fractions (Figure 5) corresponded roughly with the UV trace (Figure 4). These fractions exhibited very little absorbance at 400 nm at pH 4, but exhibited increased absorbance at 400 nm at pH 10 corresponding to the UV absorption associated with avenanthramide A, B, and C, as well as other fractions (Figure 5). Thus, the color extracted from cooked oats at alkaline pH appeared associated with the avenanthramides.

Generation of high pH by cooking with bicarbonate

It is clear that a yellow-green color will develop in oats cooked under alkaline conditions and that the green color appeared to be derived from phenolic compounds including the avenanthramides. However, results presented here suggest that a pH of over 9 would be necessary to generate significant color. It seems unlikely that any water from a tap would be as alkaline as pH 9. We have considered chemical reactions that might occur in tap water to result in a higher pH. A possibility for such a reaction involves carbonates commonly found in water, especially hard water.

Carbonate is found in water in equilibrium with bicarbonate.

$$HCO_3^- + OH^- \leftrightarrow CO_3^{-2} + H_2O$$

Relative concentrations of carbonate and bicarbonate are dependent on pH, where the pK_a of bicarbonate is 6.35 and the pK_a of carbonate is 10.3. Bicarbonate can be driven out of water by heat, according the following reaction:

$$HCO_3^- \leftrightarrow CO_2 + OH^-$$

Thus, by heating water containing bicarbonate, carbon dioxide is released as a gas and hydroxyl ion concentration increases, resulting in higher pH. We hypothesized that cooking oats in water with high carbonate/bicarbonate concentration could result in an increase in pH that could result in green color.

To test this hypothesis, we cooked oats in solutions with increasing $NaHCO_3$ concentrations. After cooking, we extracted with methanol and measured the absorbance at 400 nm and measured the pH of the solution. Increasing bicarbonate concentration resulted in increased pH of the solution after cooking and increased absorbance at 400 nm (Table 2), presumably associated with color derived from phenolic compounds.

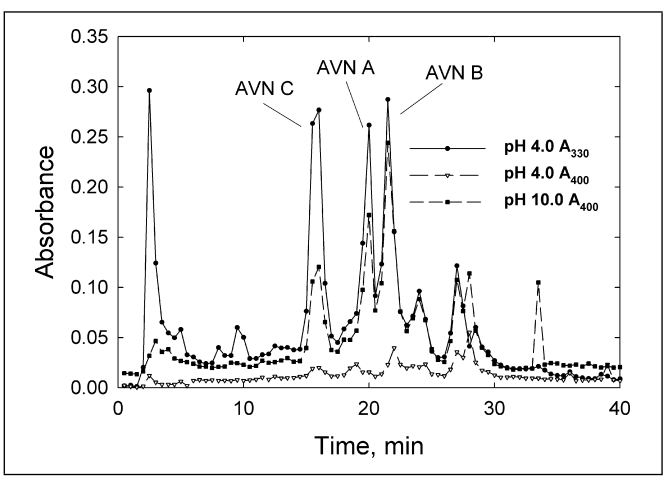

Figure 5 — Absorbencies of HPLC fractions from C-18 chromatography of oat extracts at 330 and 400 nm at pH 4 and at 400 nm at pH 10.

Our results (Table 2) indicate that cooking oats in water with bicarbonate concentration of over 50 mM can result in significantly more color (at 400 nm). We might hypothesize that the yellow-green color could develop in oats after cooking in hard water. Carbon dioxide, originating from bicarbonate driven from the water by heating could result in the increased pH that could result in color in cooked oat products.

Hard water may contain as much as 500 to 1000 mg/L bicarbonate (Van der Leeden and others 1990). This corresponds to 8 to 16 mM bicarbonate, which would not appear to be adequate to produce color during cooking according to Table 2. Reports of color generation in oats to our laboratory have been entirely anecdotal. Water obtained from locations reported to turn oats green by consumers have failed to turn oats green in our laboratory. This observation is also inconsistent with the hypothesis that hard water generates color in oats by turning alkaline during cooking.

Bicarbonate is commonly used as a leavening agent (Lajoie and Thomas 1991) in baked oat products, where concentrations of 5 to 50 mMol bicarbonate/kg of dough in a cookie formulation may not be unusual. By itself, these bicarbonate concentrations would be adequate for at least some color generation, although leavening agents containing bicarbonate are frequently blended with buffering agents such as phosphates (Chung 1992; Russell 2007), which would limit pH changes as bicarbonate is converted to CO_2 with baking.

Off colors in oat products could occur if alkaline extractions are used for industrial purposes, such as for protein extractions as are performed with sunflowers (Carter and others 1972). Neutralization of the extracts will reverse the color change. Many avenanthramides appear stable at high pH, even after heat treatments (Dimberg and others 2001).

It should also be noted that avenanthramide concentration in oats can vary widely in oats as affected by genotype and environment (Wise and others 2008). Thus, the potential for color derived from avenanthramides in alkaline conditions may vary widely among commercial supplies of oats.

Color derived from ferrous ion

Ferrous ion (Fe^{+2}) is sometimes present in well water, but is labile to atmospheric oxygen. Ferrous ion may react with components in oats in a way to generate color as described by consumers.

Steel-cut groats cooked in water containing 50 ppm Fe^{+2} developed a distinct gray to green color, primarily in the aleurone layer (Figure 6) compared to groats cooked without iron. This color could not be extracted with methanol, as was the pH dependent color (Figure 1 and 2).

Oat pastes cooked with 10 ppm Fe^{+2} or more had significant changes in their color as detected by the Minolta color meter over

Table 2 — Effect of $NaHCO_3$ concentration on the pH of oats after cooking and on the absorbance at 400 nm of the methanolic extracts from the cooked oats.

[$NaHCO_3$] mM	Absorbance 400 nm	pH (after cooking)
0	0.117	9.0
1	0.105	8.7
5	0.134	8.9
10	0.178	9.3
50	0.354	10.1
100	0.412	10.5
200	0.500	11.2
500	0.598	11.6
1000	0.567	11.6
LSD (0.05)	0.085	0.2

the pastes with no cations (Figure 7). The changes in the color dimensions as recorded by the Minolta are consistent with a green color shift. The addition of other divalent cations to oat slurries, such as Ca^{+2} or Mg^{+2} resulted in little or no color change in the slurries, especially relative to the changes observed with the Fe^{+2} ions (Figure 7).

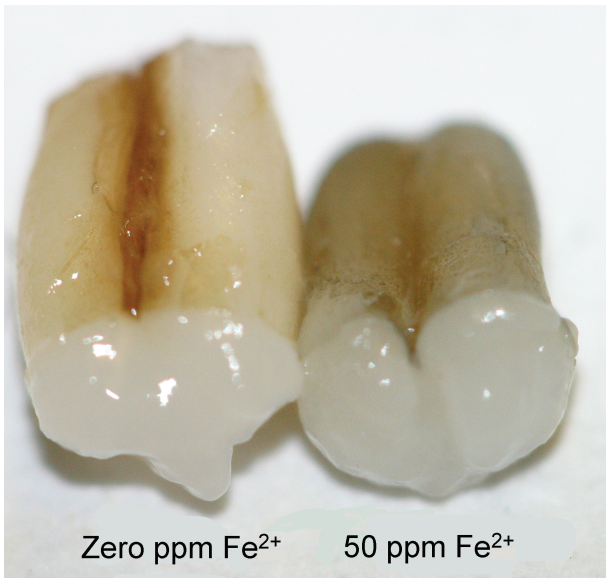

Figure 6—Color difference between steel-cut oat groats cooked in water and 50 ppm Fe^{2+} solution, showing more intense staining of the aleurone by the ferrous ion.

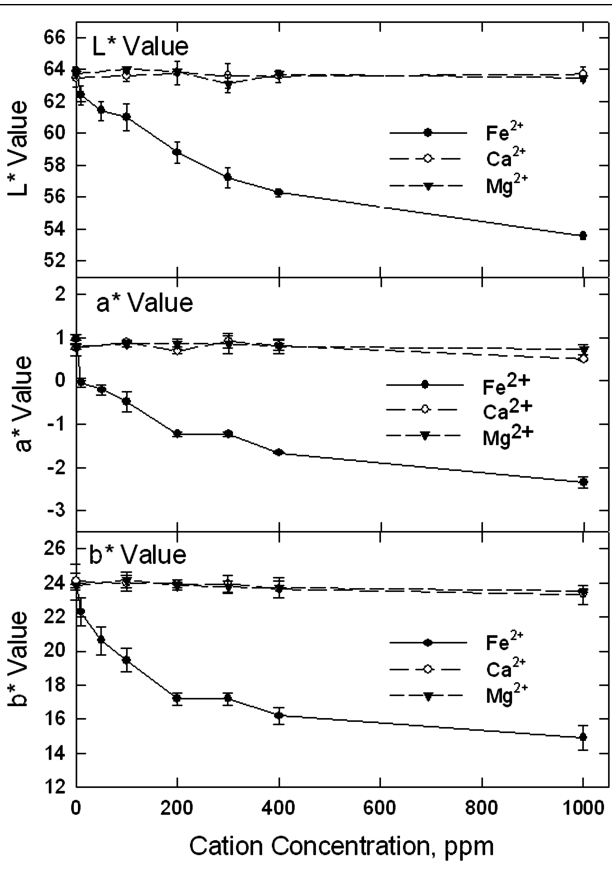

Figure 7—Effect of divalent cations on Minolta color indexes of cooked oat flour slurries.

Visually, color changes observable at 10 and 50 ppm Fe^{2+} were slight in the oat pastes (images not shown), and would be unlikely to cause a concern from a consumer. It is interesting that complaints from consumers about color developing in oats during cooking usually involve steel-cut groats. In steel-cut groats, the more intense staining of the aleurone (Figure 6) may more readily trigger a response from a consumer. Oat pastes at 200, 400, and 1000 ppm Fe^{+2} developed distinctive gray colors (Figure 7). Ferrous iron concentrations of over 50 ppm are unlikely to occur in any water supply considered suitable for human consumption. We show the response of oat color at Fe^{+2} concentrations of 200 ppm and higher to show the color changes under exaggerated conditions.

Conclusions

Our results best support the hypothesis that the green oat color observed by consumers while cooking steel-cut groats in tap water is due to a reaction of the oat aleurone with Fe^{+2} ions. This effect would only be observed with water freshly drawn from a well, because Fe^{+2} is quickly oxidized by atmospheric oxygen. Any consumer experiencing this problem would be advised to allow their water to sit in atmospheric condition for an interval before cooking their oats.

This problem would tend to be observed in steel-cut groats more readily than in oat flakes, because of the intact aleurone on the exterior of the groat would exhibit the staining more obviously than flakes.

Our results also indicate that the yellow-green color found in oat products at alkaline pH is associated with the avenanthramides. We find that bicarbonate, a common solute in tap water, can turn water alkaline when it is boiled as the bicarbonate is converted to CO_2, which escapes as a gas and leaves OH^- in solution. Although bicarbonate concentrations required to generate color are generally not present in tap water, sodium bicarbonate (baking soda) is used as a leavening agent in many baked products and because other oat processing protocols may require alkaline conditions, it is important to point out possible off colors from this mechanism as well.

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